REMARKS/ARGUMENTS

In an office action dated September 17, 2003, claims 34 through 58 were rejected. Reconsideration of the claims in view of the amendments to the claims and the following remarks is respectfully requested.

Status of the Claims

Claims 1 through 33 were previously withdrawn from examination.

Claim 34 has been amended to specify that the disinfectant, detergent, and acid are present in amounts effective to remove a smear layer on a prepared endodontic surface. Support for this amendment can be found in the specification at, *inter alia*, page 10, lines 8-12, page 11 lines 10-16 and 30-31, page 12, lines 1-5, and page 13, lines 9-13.

Claims 60 through 80 have been added, in which the components of the claimed solution are present in amounts effective to sterilize prepared surfaces or remove a smear layer on a prepared orthopaedic, tooth, or periodontal surfaces or from surfaces prepared for dental reconstruction or restoration. Support for these newly added claims may be found in the specification as originally filed at, *inter alia*, page 9, lines 12 through 24 and page 14, lines 5 through 19.

Upon entry of the foregoing amendments, claims 34 through 80 will be pending; no new matter has been added.

Summary of the Invention

The present invention is directed to irrigation solutions comprising disinfectant, detergent, and organic acid in amounts effective to remove smear layers from dental,

endodontic, periodontal or orthopaedic preparations. In one preferred embodiment, the solutions comprise doxycycline, polysorbate 80, and citric acid. The term "smear layer" is well known in the art and refers to the build-up of organic and inorganic debris that results from mechanical preparation of a tooth or bone surface. Complete or near-complete removal of such layers has long been a goal of practitioners in these fields, with varied results. The present invention, however, is the first solution to combine a disinfectant, a detergent, and an organic acid to effectively remove smear layers consistently and easily from prepared surfaces. The solution also results in sterilization of these surfaces at the same time.

While components of the present solution have previously been used individually or as part of other combinations, a disinfectant, a detergent, and an organic acid have not been used together to remove smear layers and are believed not to have been combined in proportions effective for this application. Additionally, previous solutions used to remove smear layers have been only partially effective, often leaving behind debris and bacteria that hinders the healing process for patients undergoing endodontic, dental, periodontal, or orthopaedic procedures. In contrast, the present invention effects substantially complete removal of the smear layer and sterilization of a prepared surface in a very short amount of time, an unexpected result far superior to any irrigation solution previously known these fields.

The superiority of the present invention was recognized almost immediately by experts in the endodontic field. Notably, articles describing a product made in accordance with the present invention were featured in back-to-back issues of the field's leading journal, the *Journal of Endodontics*. (See copies of articles from March 2003 and April 2003 *Journal of Endodontics*, attached.) Subsequently, seven more articles were published in the same

journal during the year 2003. Those articles demonstrated the antibacterial effectiveness and biocompatibility of the solutions described herein. Additionally, the present invention was the subject of a presentation for an audience of over 1000 endodontists at the American Association of Endodontists 2003 Annual Session. Moreover, a product in accordance with the invention has now been approved by the U.S. FDA (510(k) number K032361). Accordingly, while seemingly simple, the present invention has achieved immediate recognition.

Rejections Under 35 U.S.C. §102(b)

Claims 34 through 58 have been rejected under §102(b) in view of United States Patent No. 3,846,548 ("Akazawa"). The Examiner asserts that Akazawa "anticipat[es] and describe[es] compositions encompassed percentages of each of (A) doxycycline, (B) citric acid, and (C) polysorbate 80." (Office Action at page 3.) The Examiner admits that the cited reference does not describe use of the claimed composition for the Applicants' purpose, but "[i]t is the policy of the USPTO to give no weight to a statement of intended use in the preamble of a composition claim, as presented herein." Applicants respectfully traverse this rejection.

A claim is anticipated under §102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in the single prior art reference. *Verdegaal Bros. v. Union Oil Co. of Calif.*, 814 F.2d 628, 631 (Fed. Cir. 1987); MPEP §2131. The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 (Fed. Cir. 1989); MPEP §2131. Furthermore, to be anticipating, a reference must enable one of skill in the field of the

invention to make and use the claimed invention. *Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1378-79 (Fed. Cir. 2001).

Akazawa describes stable aqueous compositions including doxycycline, magnesium compounds, and nonionic surface active agents having a pH between 5.0 and 7.0. The compositions of Akazawa are stated to be suitable for pharmaceutical use, particularly for oral, topical, or parenteral administration. (Akazawa at column 1, lines 23-28.) Claims 34, 61, 66, 71, and 76 of the present application, the only pending independent claims, have been amended and/or added to specify that the disinfectant, detergent, and acid are present in amounts effective to remove a smear layer on a prepared endodontic, orthopaedic, tooth, or periodontal surface or from a surface prepared for dental reconstruction or restoration. Akazawa, however, does not describe effective amounts of disinfectant, detergent, and organic acid in a sterile solution for the removal of smear layers on any surfaces, let alone the surfaces of the pending claims. Additionally, Akazawa does not teach or suggest to one of skill in the art that a sterile solution of disinfectant, detergent, and inorganic acid may be used to remove the smear layer on a prepared surface in any amounts, let alone that such a combination would be effective. In fact, Akazawa only suggests the use of an organic acid in the solution described therein as a means to adjust pH to between 5.0 and 7.0. Because Akazawa lacks an element of claims 34, 61, 66, 71, and 76 and also does not enable one of skill in the art to make or use the present invention, Akazawa does not anticipate the pending claims under §102. Accordingly, Applicants respectfully request withdrawal of the rejection under §102 in view of Akazawa.

Claims 34 through 58 have also been rejected under §102(b) in view of United States Patent No. 6,383,471 ("Chen") for reasons identical to those discussed above with respect to

Akazawa.[1] Applicants respectfully traverse this rejection, as Chen does not anticipate the claimed inventions for the same reasons that Akazawa is not anticipating.

Chen describes pharmaceutical compositions including a hydrophobic therapeutic agent having at least one ionizable functional group, an ionizing agent capable of ionizing the functional group, a surfactant, and optionally solubilizers, triglycerides, and neutralizing agents. (Chen at col. 4, lines 29-37.) Chen does not, however, disclose effective amounts of disinfectant, detergent, and acid in a sterile solution for removing smear layers on prepared endodontic, orthopaedic, tooth, or periodontal surfaces or from surfaces prepared for dental reconstruction or restoration. In fact, Chen lists hundreds of ionizable hydrophobic therapeutic agents, ionizing agents, surfactants, and solubilizers, but discloses almost nothing about their suitability for any purpose, let alone for removing smear layers on prepared surfaces. Chen, therefore, contains no disclosure that would teach one of skill in the art to make or use the presently claimed invention. Because Chen lacks an element of the pending claims (effective amounts for removing smear layers on the surfaces set forth in the pending claims) and contains no disclosure sufficient to enable the claims presented herein, Chen, like Akazawa, does not anticipate the present invention under §102. Accordingly, Applicants respectfully request withdrawal of the rejection under §102 in view of Chen.

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[1] Applicants note that Chen does not appear to be prior art under §102(b), as its date of issue, May 7, 2002, is more than four months after the filing date of the present application. As Chen may, however, constitute prior art under another subsection of §102, Applicants will discuss the reference accordingly.

Rejections Under 35 U.S.C. §103(a)

Claims 34 through 58 have been rejected under §103(a) as obvious over Akazawa and/or Chen for the reasons described previously. Applicants respectfully traverse this rejection.

References may not be relied upon under §103 unless they are analogous prior art, i.e. either in the field of the applicant's endeavor or reasonably pertinent to the particular problem with which the present application is concerned. *In re Oetiker,* 977 F.2d 1443, 1446 (Fed. Cir. 1992); MPEP §2141.01(a). Applicants respectfully submit that both Akazawa and Chen are non-analogous prior art to the present invention. Akazawa and Chen are directed to pharmaceutical compositions intended for systemic administration in the body. In contrast, the present invention is directed to local, topical irrigation for the purpose of removing smear layers from prepared endodontic, orthopaedic, tooth, or periodontal surfaces or surfaces prepared for dental reconstruction or restoration and also for disinfecting these surfaces. Additionally, whereas Akazawa and Chen are directed to the field of pharmaceuticals, the present invention is directed to the fields of dentistry, endodontics, periodontics and bone reconstruction and restoration. Therefore, because Akazawa and Chen are not in the field of Applicants' endeavor or reasonably pertinent to the problem of smear layer removal, Applicants believe that they are not analogous references under §103.

Even if Akazawa and/or Chen were analogous art, they are nevertheless insufficient to establish obviousness of the present invention under §103(a). To establish a *prima facie* case of obviousness, "there must be some teaching, suggestion, or motivation in the prior art to make the specific combination that was made by the applicant." *In re Oetiker*, 24 U.S.P.Q.2d 1443, 1445 (Fed. Cir. 1992). Further, there must be a reasonable expectation of success in

achieving the intended purpose of the invention. Lastly, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Neither Akazawa nor Chen teaches or suggests a sterile solution of disinfectant, detergent, and organic acid in amounts effective to remove smear layers on prepared endodontic, orthopaedic, tooth, or periodontal surfaces or from surfaces prepared for dental reconstruction or restoration. Akazawa and Chen, therefore, fail to teach or suggest all of the limitations of the pending claims. Additionally, there is no reasonable expectation of success because one of skill in the art, when considering the disclosures of either Akazawa or Chen, would have no expectation that any of the compositions disclosed therein would be useful or effective for removing smear layers on and disinfecting the claimed surfaces.

Finally, the determination of obviousness under §103 must include evaluation of objective evidence or secondary considerations that indicate non-obviousness of an invention. These considerations include unexpected results, long-felt need, and commercial success, among others. *See Graham v. John Deere Co.*, 383 U.S. 1 (1966); MPEP §2141. Such factors are abundantly present in this invention. As discussed above, the components of the present invention have been used previously in the art, both individually and in combination with other compounds. None of the previously known irrigation solutions, however, has exhibited the superior results of the present invention, which achieves substantially complete removal of the smear layer and disinfection of prepared surfaces in a short period of time with very low toxicity. Thus, the effectiveness of the solutions claimed herein is unexpected

to those of skill in the fields of endodontics, dentistry, periodontics, and orthopaedics.

Additionally, the present invention removes substantially all of the smear layer from prepared surfaces and sterilizes those surfaces, a result that has been desired by practitioners since the existence of smear layers was first described almost 30 years ago. (See examples 1 through 6 of the specification as originally filed.) The present invention, though new to the fields described above, has also achieved great success and recognition, particularly in the field of endodontics. For example, a product in accordance with Applicants' invention was featured on the cover of the *Journal of Endodontics* in two back-to-back months and other articles were included in seven subsequent issues. It was also the subject of a presentation at the 60th Annual meeting of the American Association of Endodontists in Tampa, Florida in 2003, and has recently received FDA approval (U.S. FDA 510(k) #K032361).

Because the references do not teach or suggest all of the limitations of the present claims and do not provide an expectation of success, Akazawa and Chen are insufficient to create a *prima facie* case of obviousness of the present invention. The present invention also exhibits evidence of secondary considerations that further contribute to a conclusion of non-obviousness. Accordingly, withdrawal of the rejection of claims 34 through 58 under §103 is respectfully requested.

Conclusion

Applicants respectfully submit that the foregoing arguments and amendments place this application in condition for allowance. Applicants invite the Examiner to contact the undersigned at (215) 557-5966 to clarify any unresolved issues raised by this response.

Date: January 23, ~~2003~~ 2004

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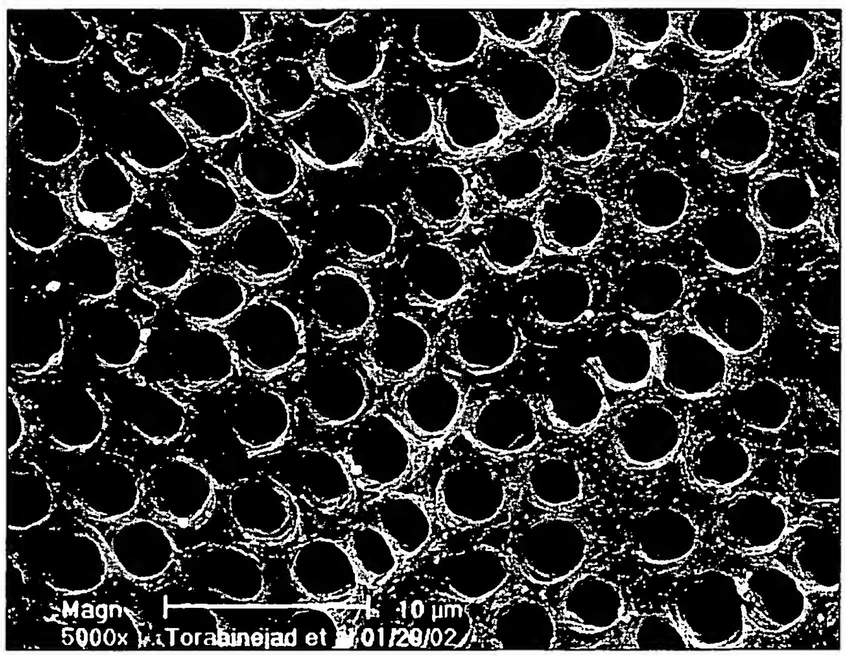

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A New Solution for the Removal of the Smear Layer

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SCIENTIFIC ARTICLES

A New Solution for the Removal of the Smear Layer

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Various organic acids, ultrasonic instruments, and lasers have been used to remove the smear layer from the surface of instrumented root canals. The purpose of this study was to investigate the effect of a mixture of a tetracycline isomer, an acid, and a detergent (MTAD) as a final rinse on the surface of instrumented root canals. Forty-eight extracted maxillary and mandibular single-rooted human teeth were prepared by using a combination of passive step-back and rotary 0.04 taper nickel-titanium files. Sterile distilled water or 5.25% sodium hypochlorite was used as intracanal irrigant. The canals were then treated with 5 ml of one of the following solutions as a final rinse: sterile distilled water, 5.25% sodium hypochlorite, 17% EDTA, or a new solution, MTAD. The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portion of each canal were examined under a scanning electron microscope. The results show that MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules when canals are irrigated with sodium hypochlorite and followed with a final rinse of MTAD.

Microscopic examinations of root canals show that they are irregular and complex systems, with many cul-de-sacs, fins, and lateral canals. Additionally, numerous dentinal tubules open onto the root canal surface. When the dental pulp undergoes pathologic changes caused by trauma or carious invasion, the root canal system becomes susceptible to infection by several species of bacteria, with their toxins and their by-products. The microorganisms present in the root canal not only invade the anatomic irregularities of the root canal system, but they also invade the dentinal tubules and can reinfect the root canals if they remain viable after inadequate root canal treatment (1).

The main objectives of root canal therapy are cleaning and shaping and then obturating the root canal system in three dimensions to prevent reinfection. Many instrumentation techniques have been proposed to shape root canals to facilitate their complete obturation. Less attention has been directed toward the ability of these techniques to completely clean and disinfect the root canal system. Studies show that currently used methods of instrumentation, especially rotary instrumentation techniques, produce a smear layer that covers root canal walls and the openings to the dentinal tubules (2, 3). The smear layer consists of organic and inorganic substances, including fragments of odontoblastic processes, microorganisms, and necrotic materials. Presence of this smear layer prevents penetration of intracanal medication into the irregularities of the root canal system and the dentinal tubules and also prevents complete adaptation of obturation materials to the prepared root canal surfaces (4).

Various organic acids, ultrasonic instruments, and lasers have been used to remove the smear layer. Based on available evidence, it seems that these agents and methods do not provide complete disinfection of the root canal spaces in all cases when used in one-visit root canal therapy. Because of the ineffectiveness of these techniques, many practitioners rely on placement of $Ca(OH)_2$ in the root canals to assist in canal disinfection (5, 6). As a result of this recommendation, root canal therapy has to be performed in more than one appointment.

In addition to acids, ultrasonic and lasers, tetracycline has been recommended as a chelating agent during periodontal and endodontic treatment. Doxycycline has been used during periodontal treatment because of its antibacterial and chelating ability as well as its substantiveness (7). Barkhordar et al. (8) and Haznedaeroglu and Ersev (9) recommended the use of tetracycline HCl to remove the smear layer from the surfaces of instrumented canals and root-end cavity preparations. However, these investigators did not examine the antibacterial effects of tetracycline when used as an intracanal irrigant. A search of the endodontic literature showed the absence of any reports regarding the ability of an irrigant capable of removing the smear layer and disinfecting the root canal system. The purpose of this study was to investigate the effect of a new irrigation solution (MTAD), containing a mixture of a tetracycline isomer, an acid, and a detergent on the surface of instrumented root canals. (A patent application has been filed covering the technology described in this article.)

TABLE 1. Solutions used during and after root canal preparation

Group (n = 12)	Irrigating solution during root canal preparation	Final solution for removal of the smear layer
A (+ Control)	Distilled water	Distilled water
B	5.25% NaOCl	5.25% NaOCl
C (− Control)	5.25% NaOCl	17% EDTA
D	5.25% NaOCl	MTAD

MATERIALS AND METHODS

Forty-eight extracted maxillary and mandibular single-rooted noncarious human teeth were used for this study. Teeth with previous coronal restorations or root canal treatment were excluded. The teeth were randomly divided into 4 groups of 12 teeth each according to the type of irrigants used during and after instrumentation (Table 1).

After preparing a conventional access preparation for each tooth, a K-type file (size 10 or 15) was used to determine the working length by penetrating the apical foramen and pulling back into the clinically visible apical foramen. The working length of each tooth was 21 to 25 mm. Each canal was instrumented using a combination of passive step-back and rotary 0.04 taper nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) (10). The apical foramen of each tooth was enlarged to a size 30 file. Sterile distilled water was used as an intracanal irrigant in 12 root canals. These teeth were used as the positive control samples. For the remaining 36 teeth, 5¼% sodium hypochlorite (NaOCl) was used as an intracanal irrigant.

To determine the effect of experimental and control solutions as a final rinse on the surface of instrumented root canals, the canals were treated with 5 ml of one of the following solutions:

A. Sterile distilled water (positive control)
B. 5¼% NaOCl
C. 17% EDTA (Roth International Ltd., Chicago, IL) (negative control)
D. MTAD, a new solution containing a mixture of a tetracycline isomer (doxycycline, Sigma-Aldrich Company, St. Louis, MO), an acid (citric acid, Sigma-Aldrich), and a detergent (Tween-80, Sigma-Aldrich)

Table 1 shows the distribution of samples. After instrumentation each canal was initially irrigated with 1 ml of one of the solutions. To ensure a uniform and direct contact of each irrigant with the root canal walls, a #15 barbed broach was wrapped with cotton and soaked with one of the solutions and placed to the working length. After 4 min, the wrapped broach was moved up and down 4 to 5 times, and then each canal was irrigated with 4 ml of one of the experimental or control solutions as a final rinse. The total exposure time to the final solution was approximately 5 min. The canals were then irrigated with 10 ml of sterile distilled water and dried with paper points. The teeth were split longitudinally, and half of each tooth was placed in a 2% glutaraldehyde solution for 24 h. The other half of each tooth was discarded. The fixed specimens were rinsed three times with a sodium cacodylate buffered solution (0.1 M, pH 7.2), incubated in osmium tetroxide for 1 h, dehydrated with ascending concentrations of ethyl alcohol (30–100%), and placed in a desiccator for at least 24 h. Each specimen was mounted on an aluminum stub and coated with 25 μm of gold-palladium and examined under a scanning electron microscope.

The specimens were then coded based on the final irrigation solution. In a blind manner, two investigators scored the presence or absence of smear layer on the surface of the root canal or in the dentinal tubules at the coronal, middle, and apical portion of each canal according to the following criteria:

1 = No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open.
2 = Moderate smear layer. No smear layer on the surface of root canal, but tubules contained debris.
3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules.

In addition, the same investigators scored the degree of erosion of dentinal tubules as follows:

1 = No erosion. All tubules looked normal in appearance and size.
2 = Moderate erosion. The peritubular dentin was eroded.
3 = Severe erosion. The intertubular dentin was destroyed, and tubules were connected with each other.

The Cochran-Mantel-Haenszel method was used to analyze the data.

RESULTS

Removal of smear layer from the surfaces of root canals revealed the presence of more abundant and larger dentinal tubules in the coronal third of root canals compared with those seen in the middle and apical thirds of the root canal system. The dentinal tubules in the apical third of the canals were smaller and fewer than those observed in the rest of the root canals (Fig. 1). In addition, removal of the smear layer showed the presence of many lateral canals in the apical thirds of the root canal systems (Fig. 2).

Examination of the surface of root canal walls in group A (positive control) showed the presence of a heavy smear layer throughout the entire length of the root canals (Fig. 3). The surfaces of samples in group B were similarly covered with a heavy layer of debris in the coronal, middle, and apical portion of each canal (Fig. 4). Dentinal tubules were not visible in groups A and B.

The surfaces of root canals and the dentinal tubules in the coronal and middle thirds of samples in group C were free of debris. Severe erosion was noted on the root canal surfaces in this group (Fig. 5). The surfaces of root canals in the apical third of the samples in group C were also free of debris, but the dentinal tubules contained moderate amounts of debris (Fig. 6). The surfaces of root canals and the dentinal tubules in the coronal, middle, and apical thirds of samples in group D were free of debris (Fig. 7).

Comparison of the four treatment groups showed a statistically significant difference in the amount of debris remaining at all three levels of the canals ($p < 0.0001$). No significant difference was found between canals treated with distilled water (group A) and canals treated with NaOCl (group B) ($p = 1$). However, the canals in groups C and D were significantly cleaner than in groups A and B ($p < 0.0001$). Comparison of remaining debris in groups C and D in the coronal and middle thirds of the canals showed no significant differences between the effects of the final irrigants (p values shown in Table 2). The dentinal tubules in the apical third of canals treated with MTAD were significantly cleaner than those treated with EDTA ($p < 0.0001$).

The amount of erosion was statistically analyzed only between groups C and D, because the smear layer was not removed in

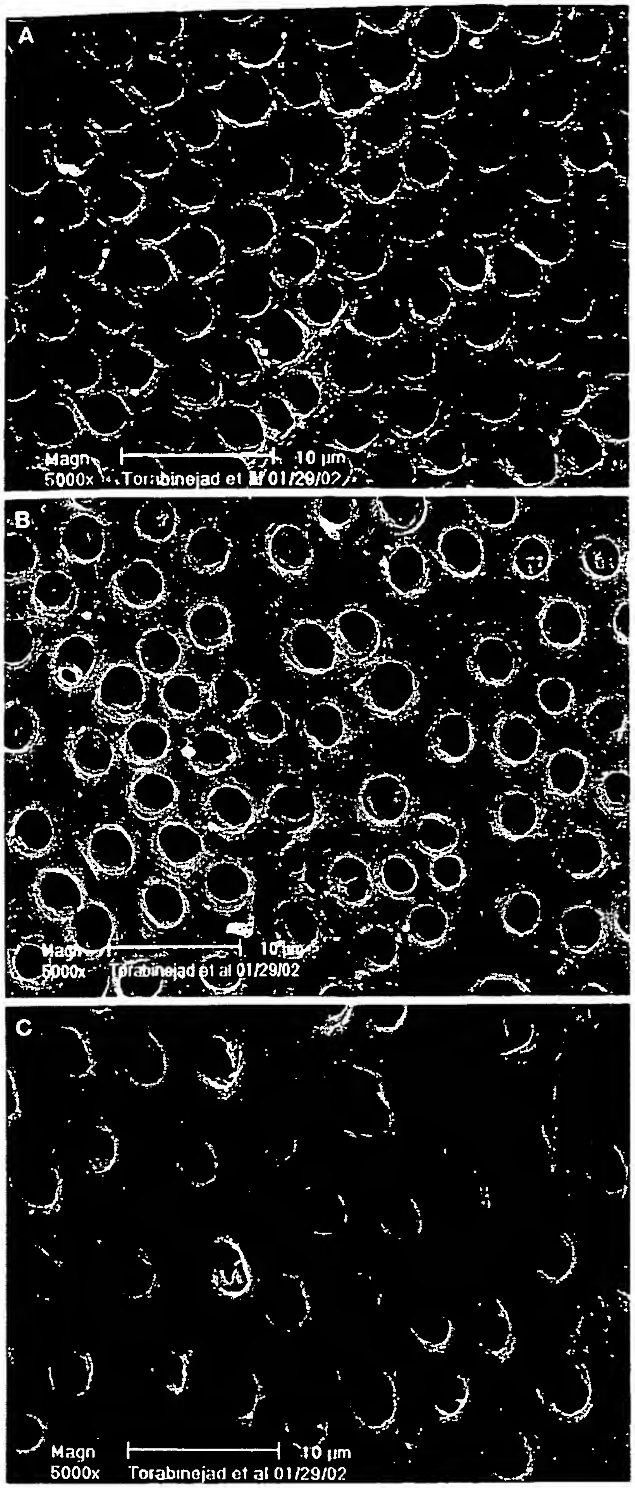

FIG 1. More and larger dentinal tubules were observed in the coronal third (A) of root canals compared with those seen in the middle (B) and apical (C) thirds. Instrumentation with 5.25% NaOCl as root canal irrigant and treatment with 5 min of MTAD as a final rinse have resulted in complete removal of the smear layer from the surfaces of the root canal (original magnification ×5000).

groups A and B (Table 3). Although the coronal and middle sections were significantly more eroded in group C than in group D (p = 0.0003 and p = 0.0005, respectively), the amount of erosion in the apical section was not statistically different (p = 0.1276) in these two groups. Furthermore, EDTA caused significantly more erosion in the coronal portion of the canals compared with the middle third of the canals (p = 0.0056).

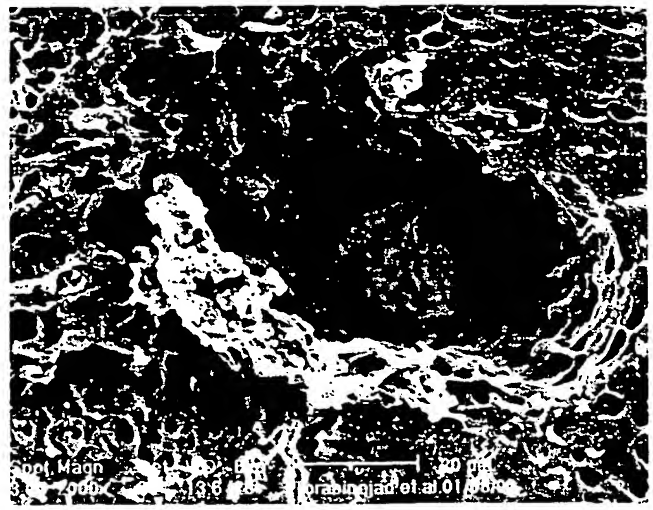

FIG 2. Removal of the smear layer from the surface of an instrumented root canal with 5.25% NaOCl as a root canal irrigant and MTAD as a final rinse have resulted in the opening of many dentinal tubules and a large lateral canal (original magnification ×5000).

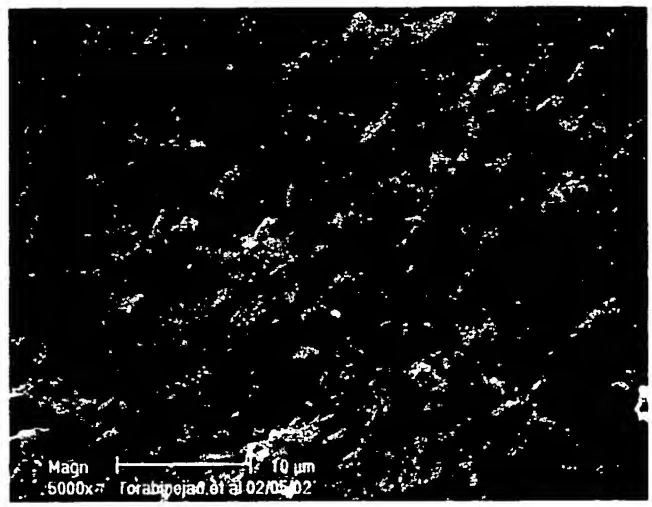

FIG 3. Presence of the smear layer on the surface of the middle of a root canal irrigated with sterile distilled water and a final rinse of the same solution (original magnification ×5000).

DISCUSSION

The main purpose of this investigation was to evaluate the effectiveness of an irrigant solution with ingredients capable of disinfecting the dentin, removing the smear layer, opening the dentinal tubules and allowing the antibacterial agents to penetrate the entire root canal system. Various antibiotics such as penicillin, bacitracin, and streptomycin have been used in the past to disinfect the root canals (11). However, because of the ineffectiveness of these antibiotics against the flora of infected root canals and their potential antigenicity, their use has been very limited. Tetracycline, including tetracycline-HCl, minocycline, and doxycycline, are

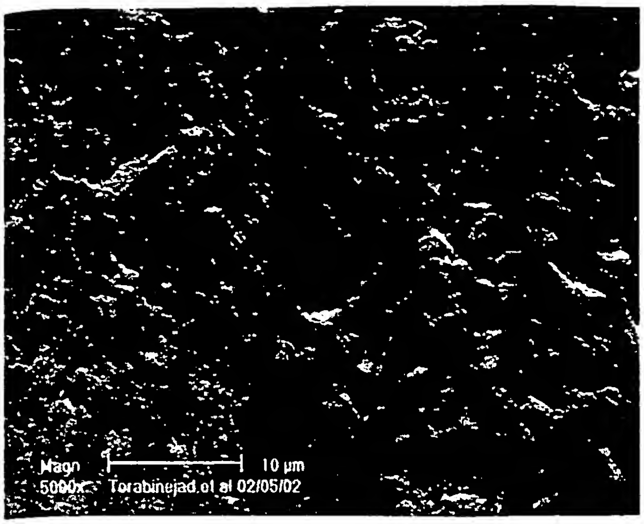

FIG 4. Presence of the smear layer on the surface of the middle of a root canal irrigated with 5.25% NaOCl and a final rinse of the same solution (original magnification ×5000).

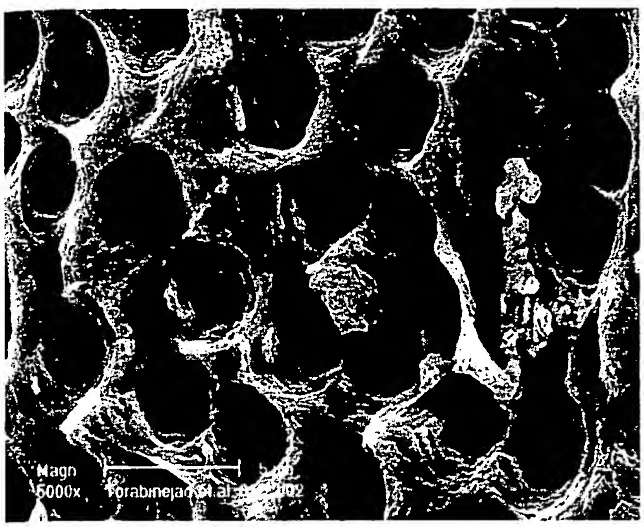

FIG 5. Sever erosion of the dentinal tubules is present in the coronal portion of a root canal treated with NaOCl as a root canal irrigant and 17% EDTA as a final irrigant for 5 min (original magnification ×5000).

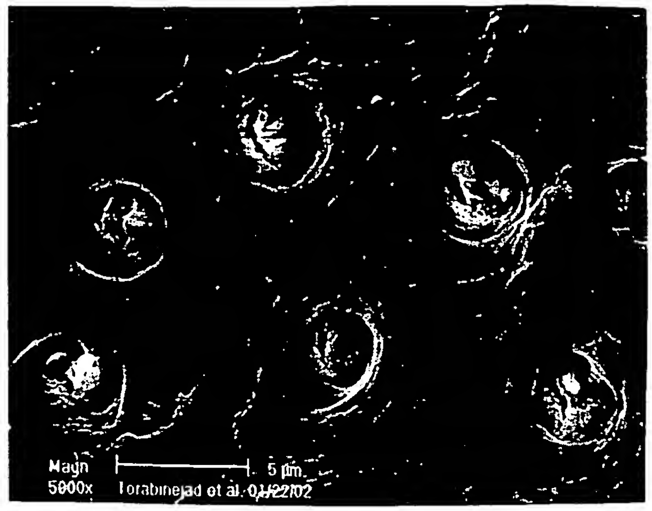

FIG 6. Instrumentation of a root canal with 5.25% NaOCl as root canal irrigant and treatment with 5 min of 17% EDTA as a final rinse have resulted in the removal of the smear layer in the apical portion of the root canal. Note presence of debris in the dentinal tubules in this region (original magnification ×5000).

broad-spectrum antibiotics that are effective against a wide range of microorganisms. Tetracycline is bacteriostatic in nature. This property may be advantageous because in the absence of bacterial cell lysis, antigenic by-products (i.e. endotoxin) are not released. Tetracycline has many unique properties other than its antimicrobial effect. It has a low pH and thus can act as a calcium chelator and cause enamel and root surface demineralization (12). Its surface demineralization of dentin is comparable to that seen using citric acid (13). In addition, it has been shown that it is a substantive medication (becomes absorbed and gradually released from tooth structures such as dentin and cementum) (13, 14). Finally, studies have shown that tetracycline significantly enhances healing after surgical periodontal therapy (7).

The effects of the tetracycline family of antibiotics on the removal of smear layer from the surface of instrumented root canals and root-end cavity preparations have also been studied (8, 9). However, these studies did not examine the antibacterial effects of tested tetracyclines when used to remove the smear layer. In a pilot study we instrumented root canals, removed the smear layer, infected the dentinal tubules with whole saliva or *Enterococcus faecalis* for 2 weeks and then irrigated the root canals with 5 ml of different concentrations of doxycycline at various time intervals. Our results showed that placement of low concentrations of doxycycline in the root canals for 5 min was an effective antibacterial agent and prevented bacterial growth in 100% of our samples. Similar attempts with penicillin and erythromycin were ineffective.

Removal of the smear layer from the surface of instrumented root canals should allow the penetration of doxycycline into the root canal irregularities and the dentinal tubules. Various chemicals have been used to remove the smear layer. They include different formulations of EDTA, acetic acid, citric acid, polyacrylic acid, tannic acid, and Bis-dequalinium-acetate (4). The reagents that reacted with doxycycline in the test tubes were eliminated before their ability to remove the smear layer was tested. In several pilot studies we treated instrumented root canals with various volumes (1–10 ml) of different concentrations of the acetic acid, polyacrylic acid, and citric acid in combination with low concentrations of doxycycline as a final rinse for different time intervals (1–10 min). Our results showed that none of the above solutions were as effective as 5 ml of a mixture of doxycycline and citric acid for 1 to 5 min in removal of the smear layer. After identification of this mixture as our combination of choice, we mixed it with different concentrations of a number of detergents to lower the surface tension and increase the penetrating ability of the irrigating solution. Experimentation with various concentrations of these materials showed that a mixture of doxycycline, citric acid, and Tween-80 was capable of removing the smear layer from the surface of instrumented root canals better than a combination of only doxycycline and citric acid. After identification of our "ideal" combination, we set up this investigation to determine the effect of this solution as a final rinse on the surface of instrumented root canals compared with that of saline (positive control), NaOCl, and EDTA (negative control).

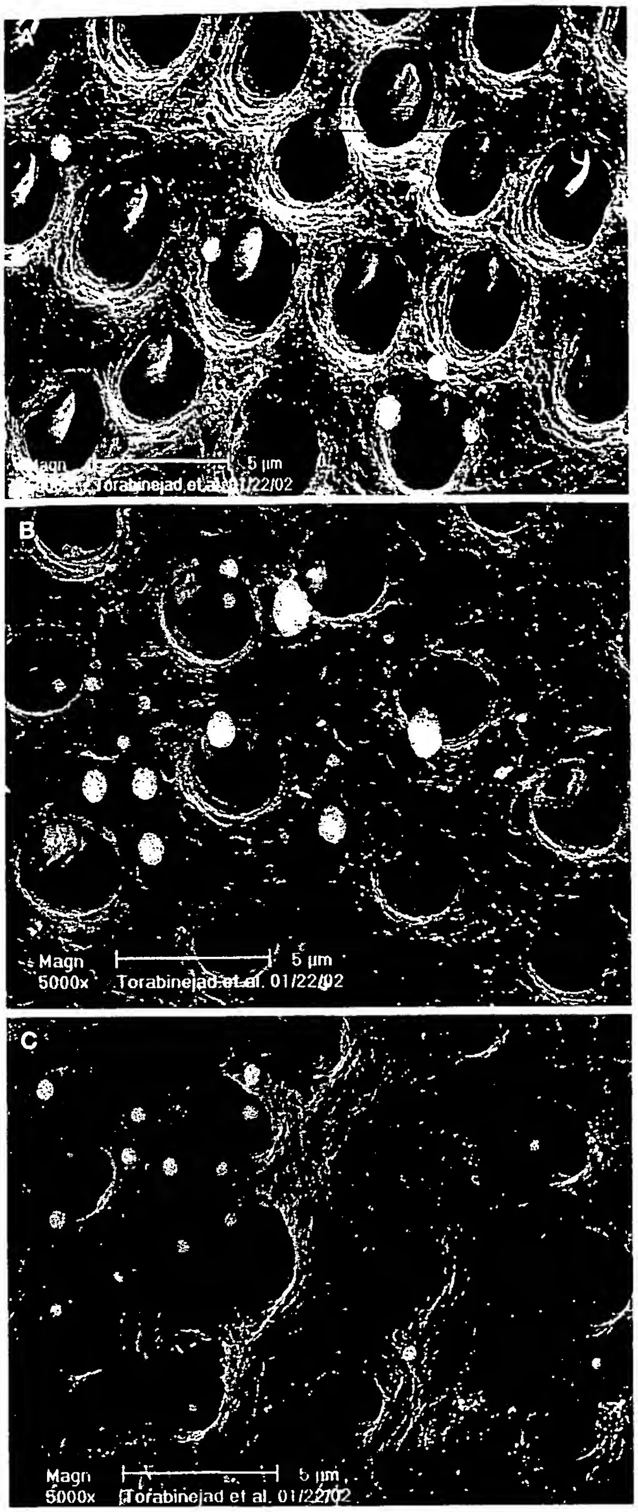

FIG 7. Instrumentation of a root canal with 5.25% NaOCl as root canal irrigant and treatment with 5 min of MTAD as a final rinse resulted in the removal of the smear layer in the coronal (A), middle (B), and apical (C) portions of the root canal (original magnification ×5000).

The teeth selected for this investigation ranged from 20 to 25 mm in length with intact clinical crowns. The entire canal length was utilized to test the efficacy of the solutions in all segments of the root including the apical third. In many previous studies the clinical crowns were removed and the effects of the test solutions

TABLE 2. P values for smear layer removal among groups A to D

Canal Level	Group Comparison	p Value
Coronal	No significant difference between groups A and B	$p = 1$
Coronal	Groups C and D were cleaner than groups A and B	$p < 0.0001$
Coronal	No significant difference between groups C and D	$p < 0.2835$
Middle	No significant difference between groups A and B	$p = 1$
Middle	Groups C and D were cleaner than groups A and B	$p < 0.0001$
Middle	No significant difference between groups C and D	$p = 0.5457$
Apical	No significant difference between groups A and B	$p = 1$
Apical	Groups C and D were cleaner than groups A and B	$p < 0.0001$
Apical	No significant difference between groups C and D	$p = 0.2289$

TABLE 3. P values for the amount of erosion between groups C and D

Canal Level	Group Comparison	p Value
Coronal	Group C had more erosion than group D	$p = 0.0003$
Middle	Group C had more erosion than group D	$p = 0.0005$
Apical	No significant difference between groups C and D	$p = 0.1276$

at different levels of the root canals were not reported. The canals in this investigation were prepared with a combination of the passive step-back technique and rotary nickel-titanium instruments. This technique is an effective method to prepare root canals with rotary instruments (15). In addition, the use of the rotary files creates a significant amount of smear layer (3). The apical portion of each canal was enlarged to a size 30 file to allow adequate cleaning and penetration of the solution to the apical third of each root canal.

Scanning electron microscopy has been used to determine the effectiveness of various irrigants to remove the smear layer. Scanning electron microscopy allows an examination of morphologic details of the surfaces of prepared root canal. Based on the results of this investigation, it seems that there was no significant difference in the ability of distilled water and NaOCl to remove the smear layer from the surfaces of instrumented root canals, because both irrigants were ineffective. In a comparison of various mixtures of NaOCl, hydrogen peroxide, EDTA, and Glyoxide to saline, Baker et al. (16) found that none of the irrigants tested were significantly more effective than saline. Based on their findings and biocompatibility of saline, these investigators recommended the use of copious amounts of saline as a root canal irrigant.

The smear layer contains organic and inorganic components (4). To remove the smear layer, irrigating solutions should dissolve both components. When EDTA is alternately used with 5.25% NaOCl, the smear layer is completely removed in the middle and coronal thirds of canal preparations, but this combination is less effective in the apical third (17). This is probably because of inadequate volume and/or penetration of the solution into the

apical portion of the canal during irrigation. Our experiment corroborated these findings and showed that correct delivery of irrigating solutions is important. The placement of MTAD with a cotton-wrapped barbed broach allows intimate contact of the solution even in the apical region of the canals and improves debridement of the entire root canal wall. In a series of pilot projects, we tested foams and brushes in conjunction with MTAD solution to clean the surfaces of instrumented root canals. Our results showed that cotton-wrapped broaches were more effective and less abrasive than similar instruments covered with bristles or foams. Studies are in progress to determine the efficacy of other techniques to carry MTAD into the apical portion of the root canals systems.

Our results demonstrated that MTAD also is less destructive to the tooth structure compared with EDTA when used as a final irrigant. Close examination of the appearance of the dentinal tubules showed higher amounts of erosion with EDTA (Fig. 5). These findings corroborate the results of a recent investigation, which reported a correlation between the erosive property of EDTA and the length of time of dentin exposure to this material (18). EDTA is an effective etchant and can remove the smear layer in conjunction with NaOCl (17). The main disadvantages of the use of EDTA include its destructive effects on coronal and middle thirds of root dentin and its limited antibacterial effects. In contrast to the destructive effects of 5-min EDTA exposure, we observed no significant dentinal erosion in a pilot project when the surface of the root canals were in contact with MTAD for periods ranging from 1 to 20 min.

Based on the results of this investigation, it seems that MTAD is an effective solution for the removal of the smear layer when used as a final rinse. It does not significantly change the structure of the dentinal tubules when used in conjunction with NaOCl as a root canal irrigant. Studies are in progress to determine the efficacy of MTAD as a root canal irrigant with and without NaOCl for removing the smear layer and completely disinfecting the root canal system.

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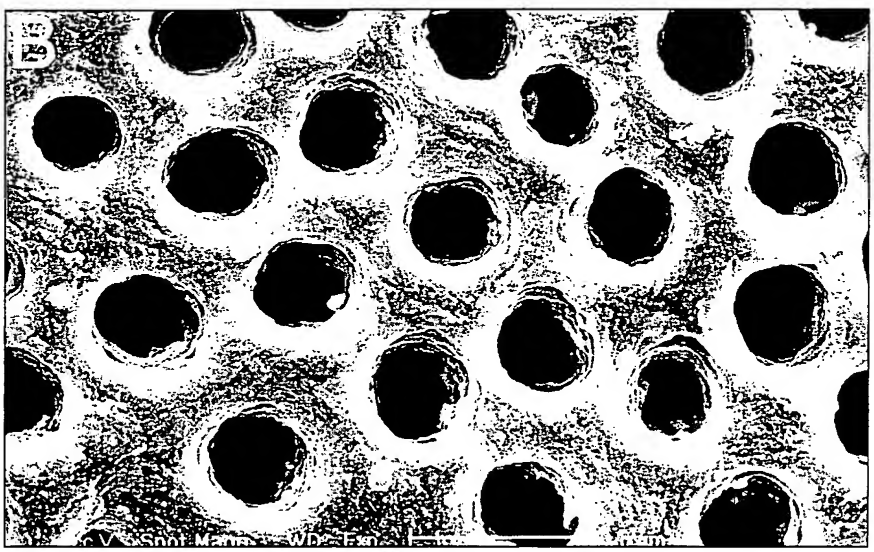

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SCIENTIFIC ARTICLES

The Effect of Various Concentrations of Sodium Hypochlorite on the Ability of MTAD to Remove the Smear Layer

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Various organic acids, ultrasonic instruments, and lasers have been used to remove the smear layer from the surface of instrumented root canals. The purpose of this study was to investigate the effect of various concentrations of sodium hypochlorite (NaOCl) as an intracanal irrigant before the use of MTAD (a mixture of a tetracycline isomer, an acid, and a detergent) as a final rise to remove the smear layer. Ten operators, using a combination of passive st p-back and rotary 0.04 taper, nickel-titanium files, prepared 80 single- and multirooted human teeth. Distilled water, four different concentrations of NaOCl, or MTAD was used as intracanal irrigant. The canals were then treated for 2 min with 5 ml of one of the following solutions as a final rinse: 5.25% NaOCl, sterile distilled water, 17% EDTA, or MTAD. The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portion of each canal were examined under a scanning electron microscope. The results show that although MTAD removes most of the smear layer when used as an intracanal irrigant, some remnants of the organic component of the smear layer remain scattered on the surface of the root canal walls. The effectiveness of MTAD to completely remove the smear layer is enhanced when low concentrations of NaOCl are used as an intracanal irrigant before the use of MTAD as a final rinse. This regimen does not seem to significantly change the structure of the dentinal tubules.

Studies have shown that current methods of cleaning and shaping root canals produce a smear layer containing inorganic and organic substances, which include fragments of odontoblastic processes, microorganisms, and necrotic materials (1–3). Ørstavik and Haapasalo (4) showed in an in vitro study the importance of the removal of smear layer and the presence of patent dental tubules in decreasing the time necessary to achieve the disinfecting effect of intracanal medications. Byström and Sundqvist (5) also have shown that the presence of a smear layer can inhibit or significantly delay penetration of antimicrobial agents, such as intracanal irrigants and medications into the dentinal tubules.

Despite controversy regarding the effect of smear layer on bacterial colonization of the dentinal tubules (6, 7) and the quality of obturation (8, 9) and because the smear layer itself can be contaminated and has the potential to protect the bacteria within the dentinal tubules, it may be prudent to remove the smear layer in teeth with infected root canals and allow disinfection of the entire root canal system (3). Various acids, ultrasonic instruments, and lasers have been used to remove the smear layer (3).

We compared the effect of a mixture of a tetracycline isomer, an acid, and a detergent (MTAD [A patent application has been filed for this technology]) as a final rinse on the surface of instrumented root canals with the effect of final rinses with sodium hypochlorite (NaOCl) or EDTA (10). The presence or absence of smear layer and the amount of erosion on the surface of the root canal walls at the coronal, middle, and apical portion of each canal in each group were examined under a scanning electron microscope (SEM). The results of that study showed that MTAD was an effective solution for the removal of the smear layer and did not significantly change the structure of the dentinal tubules when used as a final irrigant in conjunction with 5.25% NaOCl as a root canal irrigant.

The purpose of this study was to investigate the effectiveness of various concentrations of NaOCl as a root canal irrigant before the use of MTAD as a final irrigant to remove the smear layer.

MATERIALS AND METHODS

Eighty extracted maxillary and mandibular single- and multirooted human teeth were used. In multirooted teeth, the root with the largest canal was included. Teeth with previous root canal treatment were excluded. The teeth were randomly divided into

TABLE 1. Solutions used during and after root canal preparation

Group (n = 5–10)	Irrigating solution	Final rinse
A (positive control; n = 5)	Distilled water	Distilled
B (negative control; n = 5)	5.25% NaOCl	17% EDTA
C	5.25% NaOCl	5.25% NaOCl
D	Distilled water	MTAD
E	MTAD	MTAD
F	0.65% NaOCl	MTAD
G	1.3% NaOCl	MTAD
H	2.6% NaOCl	MTAD
I	5.25% NaOCl	MTAD

seven experimental groups of 10 teeth each and two control groups of five teeth each. The groups were organized according to the type of irrigants and final rinses used during and after instrumentation (Table 1).

Ten operators participated in the cleaning and shaping of the root canals. Each operator prepared one canal for groups C to I. Five operators prepared five root canals (one canal each) for group A. The other five operators prepared five root canals (one canal each) for group B. After preparing a conventional access preparation for each tooth, a K-type file (size 10 or 15) was used to determine the working length by penetrating the apical foramen and pulling back into the clinical apical foramen. The working length of each tooth was between 21 and 25 mm. Each canal was instrumented by using a combination of passive step-back and rotary 0.04 taper nickel-titanium files (Maillefer ProFile, Zurich, Switzerland) (11). The apical foramen of each tooth was enlarged to a size 30 file. Distilled water, various concentrations of NaOCl (5.25%, 2.6%, 1.3%, and 0.65%), and MTAD were used as intracanal irrigants (Table 1). One milliliter of irrigation solution was used to irrigate the root canal between each hand and rotary instrument. A total of 10 ml of irrigant was used in each root canal. The irrigants were delivered with a 27-gauge plastic needle (Ultradent Products, Inc., South Jordan, UT), which penetrated to within 1 to 2 mm from the working length in each canal. Each canal was filled with an irrigant during instrumentation. The instrumentation time for each root canal was approximately 18 to 20 min.

To determine the effect of control and experimental solutions as a final rinse on the surface of instrumented root canals, the canals were treated with 5 ml of one of the following solutions for 2 min:

1. Sterile distilled water (positive control)
2. 17% EDTA (Roth International Ltd., Chicago, IL) (negative control)
3. 5.25% NaOCl
4. MTAD, a mixture of a tetracycline isomer (doxycycline, Sigma-Aldrich Company, St. Louis, MO), an acid (citric acid, Sigma-Aldrich), and a detergent (Tween-80, Sigma-Aldrich).

Table 1 shows the distribution of samples. After instrumentation, each canal was initially irrigated with 1 ml of one of the above solutions. After 2 min, each canal was irrigated with 4 ml of one of the control or experimental solutions as a final rinse. These irrigants were delivered with a 27-gauge plastic needle (Ultradent Products), which penetrated to within 1 to 2 mm from the working length in each canal. The total exposure time for the final rinse was approximately 2 min. The canals were then irrigated with 10 ml of sterile distilled water and dried with paper points. The teeth were prepared for examination using a SEM, as described previously (10).

The specimens were coded and examined in a blind manner. Three investigators scored the presence or absence of smear layer on the surface of the root canal or in the dentinal tubules at the coronal, middle, and apical portion of each canal according to the following criteria: (10)

1 = No smear layer. No smear layer on the surface of the root canals: all tubules were clean and open.
2 = Moderate smear layer. No smear layer on the surface of root canal, but tubules contained debris.
3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules.

The same investigators scored the degree of erosion of dentinal tubules as follows:

1 = No erosion. All tubules looked normal in appearance and size.
2 = Moderate erosion. The peritubular dentin was eroded.
3 = Severe erosion. The intertubular dentin was destroyed, and tubules were connected to each other.

The Cochran-Mantel-Haenszel method was used to analyze the data.

RESULTS

Examination of the surface of root canal walls in teeth irrigated with distilled water and a final rinse of the same solution (group A) showed the presence of heavy smear layer throughout the entire length of the root canals (Fig. 1A). No smear layer was noted on the surface of most samples in the coronal and middle thirds of roots (seven of 10) in samples irrigated with 5.25% NaOCl and a final rinse of EDTA (group B). Moderate smear layer was observed on the surface of most samples in the apical thirds of the roots (four of five) in this group. Moderate to severe erosion was noted in the coronal and middle thirds of the root canal surfaces in this group (Fig. 1B). The majority of root canal surfaces (4 of 5) in the apical third of these samples had less erosion compared with the coronal and middle thirds of the canals. The surface of root canals in teeth irrigated with 5.25% NaOCl and a final rinse of the same solution (group C) were covered with a heavy layer of debris in the coronal, middle, and apical portions of each canal (Fig. 1C). Unlike group B samples, the dentinal tubules in groups A and C were not visible.

Moderate smear layer was observed on the coronal surfaces of most root canal walls of teeth (seven of 10) irrigated with distilled water and a final rinse of MTAD (group D). The same observations were made in half of the samples from the middle and apical thirds in this group. Heavy smear layer was noted on the surface of the remaining samples in these regions. Smear plugs were present in the dentinal tubules of the apical thirds of samples in this group. No erosion was noted in the dentinal tubules of group D. No smear layer was found on the coronal, middle, and apical surfaces of half of the samples (16 of 30) when root canals were irrigated with MTAD during cleaning and shaping and with the same solution as a final rinse (Fig. 2). The surfaces of the remaining samples in group E were covered with scattered remnants of the organic component of the smear layer. No erosion was seen in the dentinal tubules in this group.

Twelve of 20 samples from the coronal and middle thirds of root canals irrigated with 0.6% NaOCl and a final rinse of MTAD (group F) had no smear layer. Moderate smear layer was observed in these regions for the remaining samples. The apical thirds of roots in this group had variable levels of smear layer. No erosion

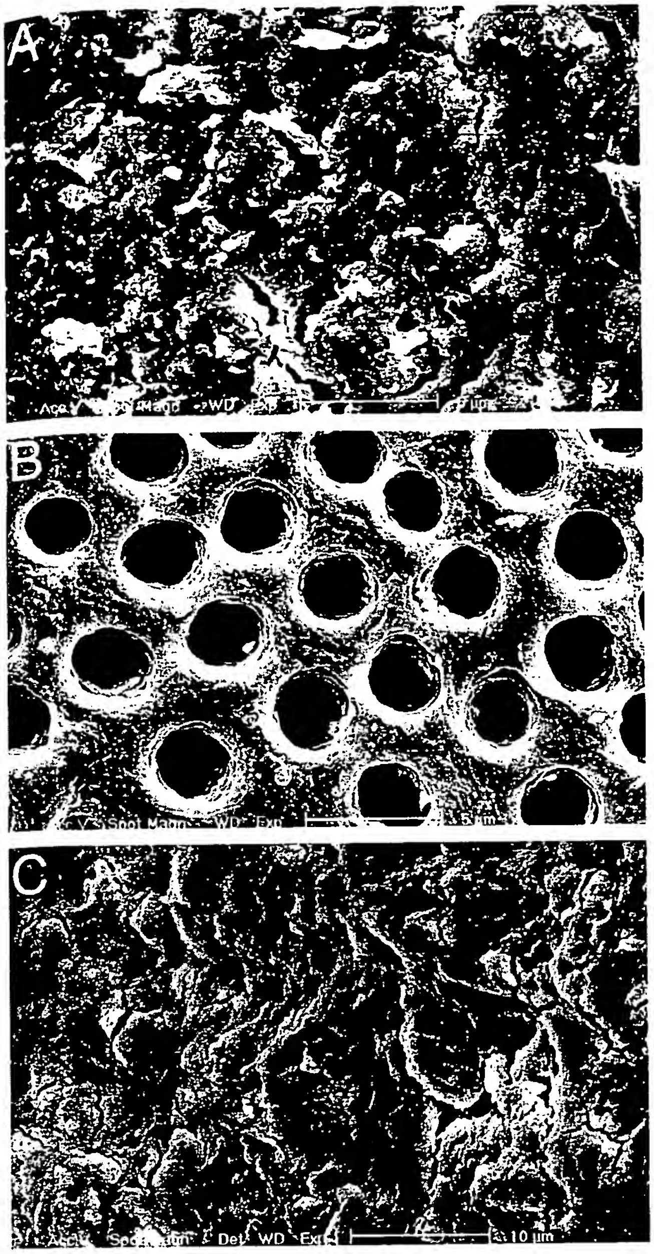

FIG 1. (*A*) Smear layer on the coronal surface of a root canal irrigated with distilled water and a final rinse of the same solution. (*B*) Moderate erosion of the dentinal tubules in the coronal portion of a root canal irrigated with 5.25% NaOCl as a root canal irrigant and 17% EDTA as a final irrigant for 2 min. (*C*) Smear layer on the surface of a root canal irrigated with 5.25% NaOCl and a final rinse of the same solution (original magnification ×5000).

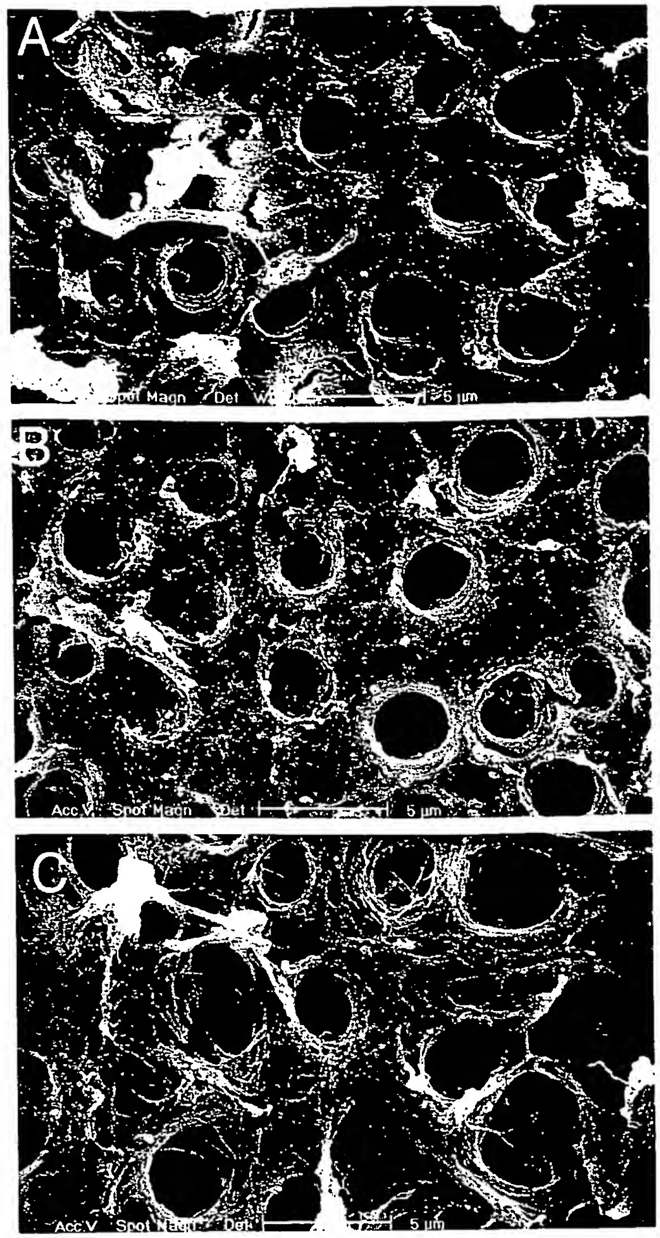

FIG 2. Instrumentation of a root canal with MTAD as root canal irrigant and the same solution as a final rinse for 2 min resulted in removal of the smear layer from the coronal (*A*), middle (*B*), and apical (*C*) levels of the root canal. Note the presence of scattered debris, which are remnants of the organic component of the smear layer (original magnification ×5000).

was seen in the dentinal tubules in group F. Most root canal surfaces (27 of 30) in the coronal, middle, and apical thirds of samples irrigated with 1.3% NaOCl and a final rinse of MTAD (group G) had no smear layer (Fig. 3). Moderate smear layer was observed in these regions for the remaining samples. Except for one sample that had moderate erosion in the coronal portion of the canal, the remaining root surfaces in group G had no erosion.

Most root canal surfaces (18 of 20) in the coronal and middle thirds of samples irrigated with 2.6% NaOCl and a final rinse of MTAD (group H) had no smear layer. Except for two samples (one with moderate smear layer and another with heavy smear layer), the remaining samples had no smear layer in apical regions of the canals. Like group G, one sample in group H had moderate erosion in the coronal portion of a root. The remaining root surfaces in this group had no erosion. All root canal surfaces (20 of 20) in the coronal and middle thirds of samples irrigated with 5.25% NaOCl and a final rinse of MTAD (group I) had no smear layer (Fig. 4). Like group G, two samples had smear layer in the apical region of the canals (one with moderate smear layer and another with heavy smear layer). The remaining samples had no smear layer in apical regions of the canals (Fig. 4). Except for two samples with moderate erosion in the coronal and middle thirds of the roots, the

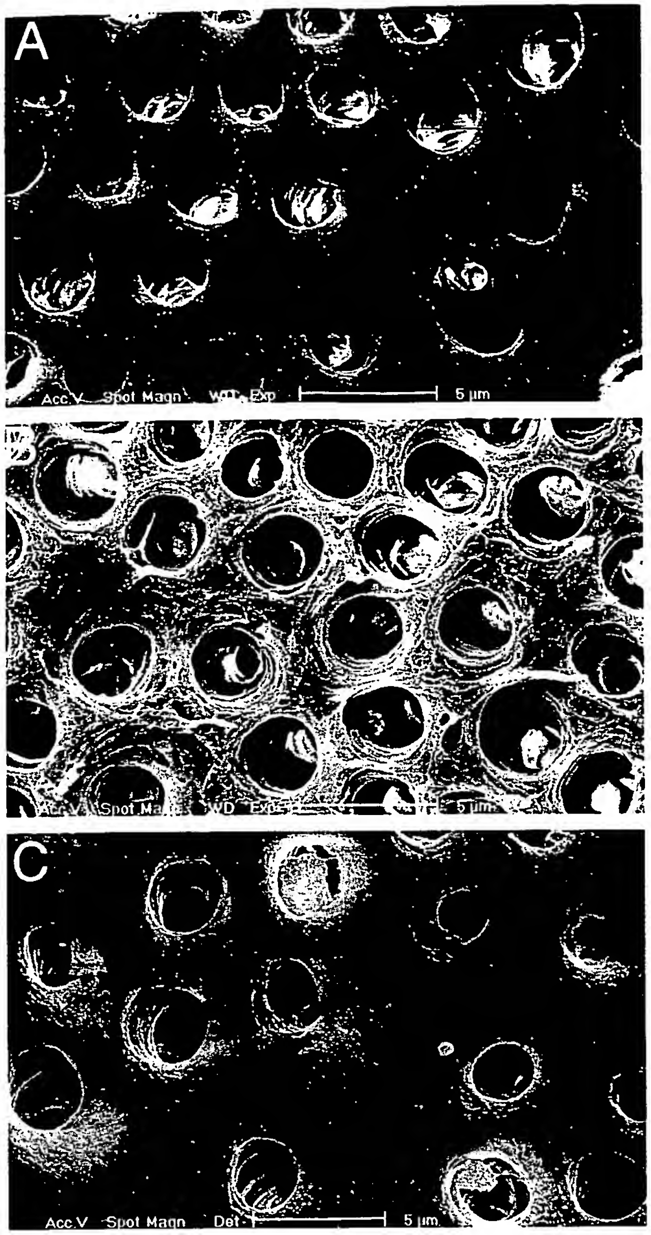

FIG 3. Instrumentation of a root canal with 1.3% NaOCl as root canal irrigant and treatment with 2 min of MTAD as a final rinse resulted in enhanced removal of the smear layer from the coronal (*A*), middle (*B*), and apical (*C*) portions of the root canal (original magnification ×5000).

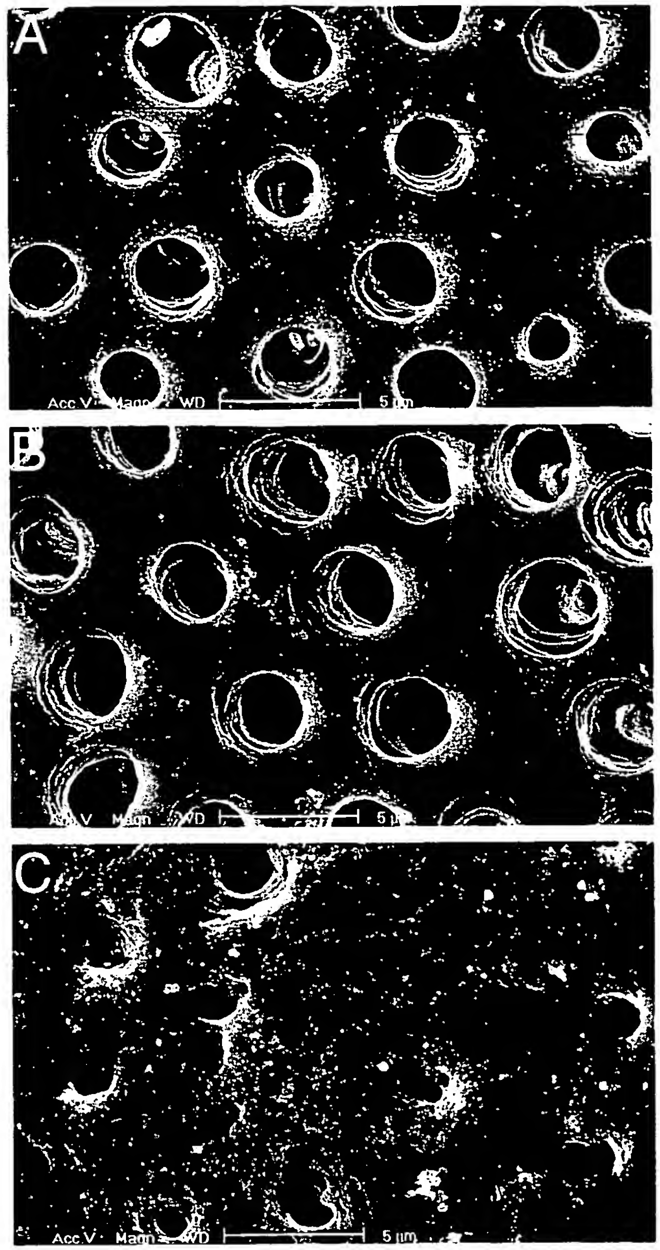

FIG 4. Instrumentation of a root canal with 5.25% NaOCl as root canal irrigant and treatment with 2 min of MTAD as a final rinse resulted in complete removal of the smear layer from the coronal (*A*), middle (*B*), and apical (*C*) surfaces of the root canal (original magnification ×5000).

remaining root canal surfaces (28 of 30) in group I had no visible erosion in the dentinal tubules.

Statistical Analysis

Table 2 shows statistical comparisons between groups A and I for the removal of the smear layer. Comparison of the nine groups showed a statistically significant difference in the amount of debris remaining at all three levels of the canals ($p < 0.0001$). No significant statistical difference was found between canals treated with distilled water (group A) and canals treated with NaOCl (group C). Root canals irrigated with 5.25% NaOCl and a final rinse of EDTA were significantly cleaner than those irrigated and rinsed with distilled water or 5.25% NaOCl.

Except for the middle thirds, root canals irrigated with distilled water and a final rinse of MTAD were significantly cleaner than those irrigated with distilled water and a final rinse of the same solution. Except for the apical thirds, the root canals irritated with 5.25% NaOCl and a final rinse of EDTA were cleaner than those irrigated with distilled water and a final rinse of MTAD. The root canals irrigated with distilled water and a final rinse of MTAD were cleaner at all levels compared with those irrigated with 5.25% NaOCl and a final rinse of the same solution. The root canals irrigated with MTAD and a final rinse of the same solution were significantly cleaner than those irrigated with 5.25% NaOCl or

TABLE 2. Pair-wise comparison of smear layer removal in groups A to I

Group	Level	B	C	D	E	F	G	H	I
A	C	B > A (0.0042)	NSD (>0.05)	D > A (0.0225)	E > A (0.0014)	F > A (0.0009)	G > A (0.0003)	H > A (0.0003)	I > A (0.0002)
	M	B > A (0.0059)	NSD (>0.05)	NSD (0.0614)	E > A (0.0009)	F > A (0.0009)	G > A (0.0002)	H > A (0.0005)	I > A (0.0002)
	A	B > A (0.0067)	NSD (>0.05)	D > A (0.0308)	E > A (0.0009)	F > A (0.0057)	G > A (0.0005)	H > A (0.0017)	I > A (0.0017)
B	C		B > C (0.0005)	B > D (0.0140)	NSD (0.1573)	NSD (0.4543)	NSD (0.6038)	NSD (0.6038)	NSD (0.1573)
	M		B > C (0.0005)	B > D (0.0069)	NSD (1)	NSD (1)	G > B (0.0379)	NSD (0.2239)	I > B (0.0379)
	A		B > C (0.0005)	NSD (0.0507)	NSD (0.1573)	NSD (0.6957)	G > B (0.0308)	H > B (0.0316)	I > B (0.0316)
C	C			D > C (0.0028)	E > C (<0.0001)	F > C (<0.0001)	G > C (<0.0001)	H > C (<0.0001)	I > C (<0.0001)
	M			D > C (0.0118)	E > C (<0.0001)	F > C (<0.0001)	G > C (<0.0001)	H > C (<0.0001)	I > C (<0.0001)
	A			D > C (0.0043)	E > C (<0.0001)	F > C (0.0007)	G > C (<0.0001)	H > C (0.0004)	I > C (0.0004)
D	C				E > D (0.0363)	F > D (0.0101)	G > D (0.0010)	H > D (0.0010)	I > D (0.0004)
	M				E > D (0.0012)	F > D (0.0101)	G > D (<0.0001)	H > D (0.0003)	I > D (<0.0001)
	A				E > D (0.0072)	NSD (0.0539)	G > D (0.0013)	H > D (0.0017)	I > D (0.0017)
E	C					NSD (0.3833)	G > E (0.0223)	H > E (0.0223)	I > E (0.0043)
	M					NSD (1)	G > E (0.0293)	NSD (0.1646)	I > E (0.0293)
	A					NSD (0.5273)	NSD (0.3415)	NSD (0.2309)	NSD (0.2309)
F	C						NSD (0.1311)	NSD (0.1311)	I > F (0.0293)
	M						G > F (0.0293)	NSD (0.1646)	I > F (0.0293)
	A						NSD (0.2466)	NSD (0.2315)	NSD (0.2315)
G	C							NSD (1)	NSD (0.3173)
	M							NSD (0.2918)	NSD (1)
	A							NSD (0.5308)	NSD (0.5308)
H	C								NSD (0.3173)
	M								NSD (0.2918)
	A								NSD (1)

> = cleaner; C = coronal; M = middle; A = apical; NSD = not statistically significant.
p values are presented in parentheses.

distilled water and a final rinse of these solutions. The same differences were observed, comparing root canals irrigated with MTAD and a final rinse of the same solution and those irrigated with distilled water and a final rinse of MTAD. No significant differences were noted when canals were irrigated with 5.25% NaOCl and a final rinse of EDTA compared to those that were irrigated with MTAD and a final rinse of the same solution. Except for the apical thirds of samples in group F, when various concentrations of NaOCl were used as irrigants and MTAD as final rinse (groups F–I), the canals were significantly cleaner at all levels compared with samples in groups A, C, and D.

No significant differences were noted between groups F (0.65% NaOCl and MTAD) and B or between groups F and E (MTAD as an irrigant and as a final rinse). When canals were irrigated with 1.3% NaOCl and a final rinse of MTAD, they were significantly cleaner in the coronal and middle thirds compared with those irrigated with MTAD and a final rinse of the same solution. Except for the middle third, which is significantly cleaner in group G (irrigated with 1.3% NaOCl and a final rinse of MTAD), there were no significant differences between the effects of treatment in groups G and F. Except for the coronal thirds in group H, no significant differences were found between middle and apical portions of canals in groups E and H. No significant differences were found between canals irrigated with 2.6%, 1.3%, or 0.65% NaOCl and a final rinse of MTAD. Except for the apical thirds of canals rinsed with MTAD and a final rinse of the same solution, the canals rinsed with 5.25% NaOCl and a final rinse of MTAD were cleaner than those in group E. The same statistical differences were found between groups F and I. No significant differences were noted between canals that were irrigated with 1.3%, 2.6%, or 5.25% NaOCl and a final rinse of MTAD.

The amount of erosion was statistically analyzed only between groups B and D to I, because the smear layer was not removed in groups A and C (Table 3). Except for the middle sections in group I, coronal and middle portions of the root canals were significantly more eroded in group B compared with groups E to I. Statistical analysis of the data demonstrated a significant difference in the amount of erosion noted in the coronal portions of the canals compared with those in the middle thirds ($p < 0.0001$). No other significant differences were found between the amounts of erosion observed between the remaining groups.

DISCUSSION

Current endodontic instrumentation methods produce a smear layer that covers the root canal surfaces. The smear layer contains inorganic and organic substances, which include fragments of odontoblastic processes, microorganisms, their by-products, and necrotic materials (12). Because of its potential contamination and adverse effect on the outcome of root canal therapy, it seems reasonable to suggest removal of the smear layer for disinfection of the entire root canal system. Current methods of smear layer removal include chemical, ultrasonic, and laser techniques—none of which are totally effective or have received universal acceptance (3, 10). The purpose of this series of investigations was to develop a regimen with components capable of disinfecting the root canal system by removing the smear layer, opening the dentinal tubules, and allowing the antibacterial agents to penetrate the entire root canal system. In a previous study, we showed that MTAD is an effective solution for the removal of the smear layer and does not significantly change the structure of the dentinal tubules when used as a final irrigant in conjunction with 5.25% NaOCl as a root canal irrigant (10). The purpose of the current study was to determine the effectiveness of various concentrations of NaOCl as root canal irrigant before the use of MTAD as a final irrigant to remove the smear layer. Various combinations of experimental groups were

TABLE 3. Pair-wise comparison of erosion levels

		D	E	F	G	H	I
B	C	B > D (0.0009)	B > E (0.0009)	B > F (0.0009)	B > G (0.0026)	B > H (0.0026)	B > I (0.0026)
	M	B > D (0.0302)	B > E (0.0302)	B > F (0.0302)	B > G (0.0302)	B > H (0.0411)	NSD (0.1149)
	A	NSD (0.1160)	NSD (0.1160)	NSD (0.1423)	NSD (0.1160)	NSD (0.1423)	NSD (0.1160)
D	C		NSD (1)	NSD (1)	NSD (0.3173)	NSD (0.3173)	NSD (0.3173)
	M		NSD (1)	NSD (1)	NSD (1)	NSD (1)	NSD (0.3173)
	A		NSD (1)	NSD (1)	NSD (1)	NSD (1)	NSD (1)
E	C			NSD (1)	NSD (0.3173)	NSD (0.3173)	NSD (0.3173)
	M			NSD (1)	NSD (1)	NSD (1)	NSD (0.3173)
	A			NSD (1)	NSD (1)	NSD (1)	NSD (1)
F	C				NSD (0.3173)	NSD (0.3173)	NSD (0.3173)
	M				NSD (1)	NSD (1)	NSD (0.3173)
	A				NSD (1)	NSD (1)	NSD (1)
G	C					NSD (1)	NSD (1)
	M					NSD (1)	NSD (0.3173)
	A					NSD (1)	NSD (1)
H	C						NSD (1)
	M						NSD (0.3428)
	A						NSD (1)

> = cleaner; C = coronal; M = middle; A = apical; NSD = not statistically significant.
p values are presented in parentheses.

compared with the effects of distilled water (positive control) and EDTA (negative control).

Similar to our previous investigation (10), the teeth selected for this study ranged from 21 to 25 mm in length with intact clinical crowns. The entire canal length was utilized to simulate clinical situations and to test the efficacy of the solutions in all segments of the root canal system. In addition, in this investigation 10 operators performed cleaning and shaping of root canals. This design also allowed simulation of clinical situations better than using one or two operators to perform clinical procedures. Unlike the procedure in our previous investigation (10), we used 2 min of final rinse instead of 5 min. This was based on our pilot projects, which showed that irrigation of canals with 5.25% NaOCl during instrumentation and 2 min of final rinse with MTAD was adequate to remove the smear layer from the surface of instrumented root canals. This exposure time may have to be adjusted after bacteriologic tests.

A scanning electron microscope was used to assess the effectiveness of various irrigants and final rinses to remove the smear layer and the amount of erosion caused in the dentinal tubules. The results show no significant difference in the ability of distilled water and 5.25% NaOCl to remove the smear layer from the surfaces of instrumented root canals, because both irrigants were ineffective. These findings are similar to those observed in our previous investigation (10), which also showed that these irrigants are not able to remove organic and inorganic components of the smear layer. Yamada et al. (13) and Baumgartner and Mader (14) showed that alternating the use of EDTA and NaOCl is an effective method for smear layer removal. We obtained similar results when 5.25% NaOCl was used as an irrigant during cleaning and shaping and EDTA as a final rinse (negative control group) for 2 min; the smear layer was completely removed from the middle and coronal thirds of canal preparations. This combination seemed less effective in the apical third of the canals. This finding, which is similar to our previous findings (10), is probably because of inadequate volume or penetration of the solution into the apical portion of the canal during irrigation.

In contrast to the procedure in our previous investigation (10), we did not use a cotton-wrapped barbed broach for an intimate contact of the solution to the entire root canal walls. In this study we used narrow plastic needles to deliver the irrigants into the apical thirds of the root canals. Opening the apical foramen of each tooth to a size 30 file and free flow of irrigant through the apical end of the root canal may have allowed more cleaning of the apical portions of root canals in this experiment. Studies are in progress to determine the efficacy of various other techniques to carry MTAD into the apical portion of the root canal systems with closed apexes.

Examination of the surfaces of the root canal walls in group B showed erosion of the dentinal tubules as a result of application of EDTA as a final rinse. Our results demonstrated that MTAD is less destructive to the tooth structure compared with EDTA when used as a final irrigant. These findings corroborate the results of our previous study (10) and another investigation (15), which reported a correlation between erosive property of EDTA and the length of dentin exposure to this solution. Based on the results of these studies, it seems that EDTA is destructive in the coronal and middle thirds of root canals if in contact with the root dentin for more than 1 min.

A comparison of the results from group D (distilled water as an irrigant and MTAD as a final rinse), group A (distilled water as an irrigant and as a final rinse), group C (5.25% NaOCl as an irrigant and as a final rinse), and group E (MTAD as an irrigant and as a final rinse) shows that MTAD dissolves inorganic and some organic components in the smear layer. The amount of smear layer in root canals irrigated with MTAD throughout cleaning and shaping is significantly less than those irrigated with 5.25% NaOCl or distilled water alone. This also was true when the surfaces of root canals from group E were compared with those in group D. The use of MTAD as a root canal irrigant left some odontoblastic processes in the dentinal tubules and organic debris on the surfaces of instrumented root canal walls. These results are similar to those reported by Yamada et al. (13) and Baumgartner and Mader (14), who showed that the use of EDTA alone removes the inorganic portion and leaves an organic layer on the canal wall. MTAD is an acidic solution with a pH of 2.15 that is capable of removing inorganic substances. Studies to determine the effect of MTAD and

other commonly used irrigants on bovine pulp and dentin are in progress.

Baumgartner et al. (16) evaluated the amount of superficial debris and the smear layer that remained on the canal wall after a combination of NaOCl and 50% citric acid. They showed that citric acid or a combination of NaOCl and citric acid irrespective of the order in which they were used was more effective than NaOCl alone to remove the smear layer from the surface of instrumented canals. In contrast to these findings, Yamada et al. (13) found that a final flush with EDTA followed by NaOCl was an effective method to remove the smear layer and superficial debris from the surfaces of instrumented root canals.

In a pilot study in extracted teeth, we used MTAD as a root canal irrigant before the use of various concentrations of NaOCl (5.25%, 2.6%, 1.3%, and 0.65%) as final rinses. During application of the final rinses, we noted a chemical reaction between NaOCl and the residual MTAD in the root canals. This reaction, which resulted in formation of a brown solution in the root canals, may be caused by the dentinal absorption and release of doxycycline present in MTAD solution and the final rinse of NaOCl. SEM examinations of the surfaces of root canals treated with these regimens showed the presence of severe erosion at all levels of the root canal. Based on these findings, it seems that MTAD reacts with the surface of dentin differently compared with citric acid or EDTA and should be used as the final rinse in conjunction with NaOCl.

Baumgartner and Cuenin (17) examined, using SEM, instrumented and uninstrumented surfaces in the middle third of root canals after the use of several concentrations of NaOCl (5.25%, 2.5%, 1.0%, and 0.5%). A smear layer with some exposed dentinal tubules was seen on all instrumented surfaces of canals regardless of the concentration of NaOCl. Our results in group C are consistent with their findings (17) and show that a chelating agent is needed to completely remove the smear layer. Comparing the results from group E (MTAD as an irrigant and as a final rinse) with groups F to I (various concentration of NaOCl as an irrigant and MTAD as a final rinse), it seems that NaOCl is needed as an irrigant to assist MTAD to completely remove the smear layer. This becomes more evident as the concentration of NaOCl increases from 0.65% to 5.25%. There is no significant difference between the ability of MTAD alone or 0.65% NaOCl as a root canal irrigant to remove the organic portion of the smear layer and MTAD as a final rinse to remove the inorganic portion of the smear layer. However, the effectiveness of NaOCl to remove the organic part of the smear layer becomes evident and significant at higher concentrations (1.3–5.25%). These findings corroborate the results of a study by Hand et al. (18), who showed that dilution of 5.25% NaOCl results in a significant decrease in the ability of this solution to dissolve necrotic tissue. Because high concentrations of NaOCl are more toxic than diluted solutions and because our results show no significant differences between the ability of 1.3%, 2.6%, and 5.25% as root canal irrigants with MTAD as a final rinse to remove the smear layer, it seems prudent to use the lowest concentration of NaOCl (1.3%) during instrumentation, followed by MTAD as a final rinse to remove the smear layer.

Based on the results of this investigation, it seems that although MTAD removes most of the smear layer when used as an intracanal irrigant, some remnants of the organic component of the smear layer remain scattered on the surface of the root canal walls. The effectiveness of MTAD to completely remove the smear layer is enhanced when low concentrations of NaOCl are used as an intracanal irrigant before the use of MTAD as a final rinse. This combination and sequence do not seem to significantly change the structure of the dentinal tubules.

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